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Low-dose pancreatic polypeptide inhibits food intake in man

David R. Jesudason, Mariana P. Monteiro, Barbara M. C. McGowan, Nicola M. Neary, Adrian J. Park, Elena Philippou, Caroline J. Small, Gary S. Frost, Mohammad A. Ghatei and Stephen R. Bloom*

Department of Metabolic Medicine, Faculty of Medicine, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK

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Pancreatic polypeptide (PP) is a gut hormone released from the pancreas in response to food ingestion and remains elevated for up to 6 h post-prandially. Plasma levels are elevated in patients with pancreatic tumours. An intravenous infusion of PP has been reported to reduce food intake in man, suggesting that PP is a satiety hormone. We investigated whether a lower infusion rate of PP would induce significant alterations in energy intake. The study was randomised and double-blinded. Fourteen lean fasted volunteers (five men and nine women) received 90 min infusions of PP (5 pmol/kg per min) and saline on two separate days. The dose chosen was half that used in a previous human study which reported a decrease in appetite but at supra-physiological levels of PP. One hour after the end of the infusion, a buffet lunch was served and energy intake measured. PP infusion was associated with a significant 11 % reduction in energy intake compared with saline (2440 (SE 200) *v.* 2730 (SE 180) kJ; $P<0{\cdot}05$). Preprandial hunger as assessed by a visual analogue score was decreased in the PP-treated group compared to saline. These effects were achieved with plasma levels of PP within the pathophysiological range of pancreatic tumours.

Pancreatic polypeptide: Energy intake: Satiety

A considerable amount of research has recently been published on circulating hormones that convey satiety and hunger to the brain and regulate energy homeostasis. Pancreatic polypeptide (PP) is a member of the peptide family that includes neuropeptide Y and peptide YY (Moran, 2003). The amount of PP released is dependent on the digestive state, with a low rate in the fasted state and a marked increase through all phases of digestion (Katsuura *et al.* 2002). Peripheral administration of PP to rodents has been found to decrease food intake significantly in a dose-dependent manner (Asakawa *et al.* 1999). Human intravenous infusions of PP (10 pmol/kg per min), which achieved supra-physiological PP plasma levels, have been reported to reduce appetite at a buffet meal and to sustain food inhibition for 24 h (Batterham *et al.* 2003).

We wished to investigate whether lower infusion rates of human PP would induce satiety and significantly reduce appetite and energy intake in man.

Methods

The study was approved by the Riverside Research Ethics Committee (under reference number 2003/3325) and run according to the principles of the declaration of Helsinki. The subjects gave informed written consent to participate in the study. Criteria for exclusion included smoking, substance abuse, pregnancy, medication (except for the oral contraceptive pill), medical or psychiatric illness, and abnormalities detected on physical examination and screening blood tests. Subjects were screened by a dietitian using the Dutch Eating Behaviour Questionnaire (Uhe *et al.* 1992) to ensure normal eating behaviour. Assessment of food palatability was carried out as for previous human appetite studies at Imperial College (Cohen *et al.* 2003). Fourteen lean volunteers (five men and nine women) of age 28·5 (SE 1·8) years and BMI 22·3 (SE 0·5) kg/m^2 were enrolled. The study was randomized, placebo-controlled and double-blinded. Each subject received both saline and PP (5 pmol/kg per min) in random order at least 3 d apart.

Subjects were fasted from 20.30 hours on the evening before infusions. The infusion started at 09.30 hours (t_0) and ran until 11.00 hours (t_{90}). At 12.00 hours (t_{150}), 1 h after the end of the infusion, a pre-weighed excess quantity of pasta buffet lunch was served. At 12.30 hours (t_{180}), the remaining food was removed and weighed. Blood samples were collected every 30 min into lithium-heparin tubes containing 5000 Kallikrein inhibitor units (0·2 ml) aprotinin (Trasylol; Bayer, West Haven, CT, USA), immediately centrifuged and the plasma was separated and frozen at −20°C (Cohen *et al.* 2003). Subjective hunger and nausea were assessed every 30 min using 100 mm visual analogue scores (VAS) (Flint *et al.* 2000). Subjects were allowed home at 14.00 hours (t_{270}) and asked to keep a food diary for 24 h. These were analysed by a dietitian, unaware of the infusion orders, using the Dietplan program (Forestfield Software Ltd, Horsham, West Sussex, UK).

Plasma PP, peptide YY, GLP-1 and total ghrelin (acylated and desacylated ghrelin) were measured using established

Abbreviations: PP, pancreatic polypeptide; VAS, visual analogue score.

* **Corresponding author:** Prof. S. R. Bloom, fax +44 (0)20 8383 3142, email s.bloom@imperial.ac.uk

in-house RIA (Adrian *et al.* 1976, 1985; Kreymann *et al.* 1987; Patterson *et al.* 2005).

Human PP was obtained from Bachem (St Helens, Merseyside, UK). The peptide was sterile following 7 d laboratory culture and the absence of endotoxin was confirmed using the limulus amoebocyte lysate assay test (Cohen *et al.* 2003).

Energy intake and plasma hormone concentrations were compared between PP and saline days using paired *t* tests. VAS were analysed using a two-way ANOVA with *post hoc* Bonferroni test. $P<0{\cdot}05$ was considered to be statistically significant.

Results

Plasma PP increased from a baseline level of 17·2 (SE 2·3) pmol/l to a peak level of 184·4 (SE 29·7) pmol/l during PP infusion. Prior to the buffet meal (60 min after the end of the infusion; t_{150}), there was no significant difference in plasma PP between PP and saline infusion days [18·5 (SE 2·6) pmol/l (saline infusion) *v.* 22·8 (SE 2·6) pmol/l (PP infusion); NS; Fig. 1(a)]. Following the meal (t_{180}), plasma PP increased similarly on both infusion days [83·1 (SE 18·9) pmol/l (saline infusion) *v.* 87·3 (SE 19·4) pmol/l (PP infusion)]. Analysis of the VAS demonstrated that the PP infusion significantly attenuated the rise in hunger before lunch [change from baseline: saline 13·6 (SE 10·6) mm, PP −8·0 (SE 11·9) mm; $P<0{\cdot}05$; Fig. 1(b)]. There were no differences in nausea as assessed by VAS between infusions (data not shown).

The PP infusion reduced energy intake during the buffet meal by 10·5 (SE 4·0) % [2730 (SE 180) kJ (saline infusion) *v.* 2440 (SE 195) kJ (PP infusion); $P<0{\cdot}05$; Fig. 2]. Analysis of the food diaries did not reveal any significant changes in energy intake over the following 24 h [5120 (SE 550) kJ (saline infusion) *v.* 4900 (SE 970) kJ (PP infusion); NS]. There were no significant differences in pulse rate or blood pressure between the two infusions (data not shown), and no side-effects were reported or observed in the study. As previously reported (Batterham *et al.* 2003), plasma ghrelin [653 (SE 79) pmol/l (saline infusion) *v.* 644 (SE 90) pmol/l (PP infusion), at t_{90}; NS], peptide YY [19·8 (SE 4·23) pmol/l (saline infusion) *v.* 15·7 (SE 3·53) pmol/l (PP infusion), at t_{90}; NS] and GLP 1 [15·8 (SE 3·45) pmol/l (saline infusion) *v.* 16·6 (SE 3·50) pmol/l (PP infusion), at t_{90}; NS] were unaffected by the PP infusion.

Discussion

It has been known for almost 30 years that pancreatic polypeptide is released in man after a meal and that levels may remain elevated for up to 6 h (Adrian *et al.* 1976, 1977). In pancreatic tumours such as glucagonomas, insulinomas and VIPomas, elevated plasma levels of PP of over 240 pmol/l have been reported (Polak *et al.* 1976). In the current study, the peak

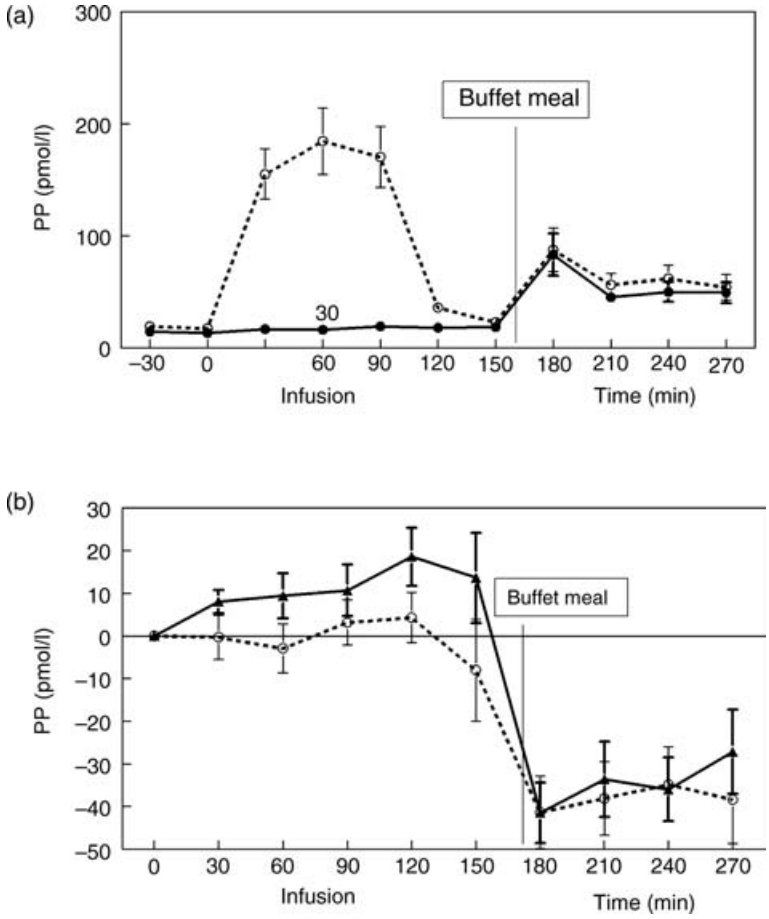

Fig. 1. Plasma pancreatic polypeptide (PP) levels (a) and visual analogue scores (b) for hunger with saline (●) and PP (○, 5 pmol/kg per min) infusions. For details of procedures, see pp. 426–427. Values are means with their standard errors depicted by vertical bars.

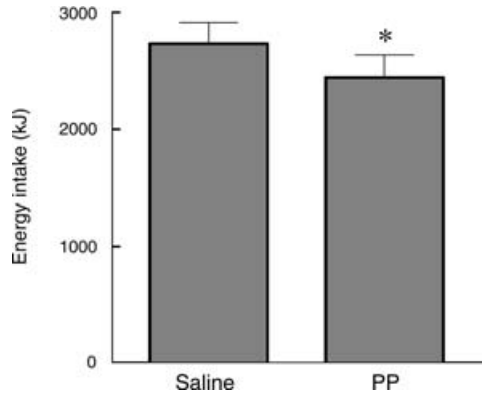

Fig. 2. Energy intake (kJ) at the buffet meal following saline and pancreatic polypeptide (PP) infusions. For details of procedures, see pp. 426–427. Values are means with their standard errors depicted by vertical bars. Mean values were significantly different from those of the saline infusion: $^{*}P<0{\cdot}05$.

levels of PP achieved were well within this range. Interestingly, at the time the buffet meal was served, 60 min after the end of the infusion, plasma PP levels had returned to baseline.

We observed an 11 % reduction in energy intake at the buffet meal following infusion of PP at 5 pmol/kg per min compared with saline control, together with a decrease in hunger as analysed by VAS. A previous human study with a higher infusion rate of PP (10 pmol/kg per min; Batterham *et al.* 2003) resulted in a decrease in hunger and a 22 % reduction in energy intake. However, this effect was achieved with distinctly higher plasma PP concentrations. In conclusion, the results suggest a dose–response relationship between increasing plasma PP and degree of energy intake reduction at a meal.

Circulating PP may inhibit food intake through both central and peripheral mechanisms. There is evidence of a central action on the Y4 receptor in the brainstem and hypothalamus (Bard *et al.* 1995). PP has also been reported to reduce gastric emptying in rodents (Asakawa *et al.* 2003). Previous studies using PP from non-primate species in man have not shown a significant alteration in gastric emptying (Adrian *et al.* 1976). Recently, Schmidt *et al.* (2005) administered human PP to lean male and female volunteers and reported a significant reduction in gastric emptying at a dose of 2·25 pmol/kg per min. Thus both central and peripheral effects may have contributed to the reduction in energy intake observed following PP infusion in the present study.

In the study by Schmidt *et al.* (2005) no change in appetite (hunger, desire to eat, satiety and prospective consumption) and food intake were observed. However, the protocol for their study was different and not directly comparable to the present study and that of a previous human study (Batterham *et al.* 2003) as the PP was infused during the meal and the VAS assessed 10 min before and up to 180 min after the meal.

In our current and previous PP human infusion study (Batterham *et al.* 2003), a decrease in food intake was achieved despite PP levels being back to normal at the start of the meal between the two groups. PP acts via hypothalamic circuits known to be involved in appetite pathways by affecting the expression of potent orexigenic peptides such as neuropeptide Y (Asakawa *et al.* 2003). The delay between hormonal signalling and gene expression may therefore provide an explanation for the effect on energy intake once PP levels have returned back to normal.

It may be possible that PP works synergistically at lower concentrations together with other appetite hormones such as peptide YY. Whilst we did not detect a significant effect of PP on ghrelin in present study consistent with previous work (Batterham *et al.* 2003), future studies looking at synergistic effects on appetite with other satiety hormones would be of interest. Our results were obtained with levels mimicking pathophysiological levels of PP. It is possible that some of the loss of appetite associated with pancreatic tumours could be explained by elevated concentrations of circulating PP.

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